International Application No. PCT/JP00/06129



Date: March 7, 2002

Page 1

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) **CONCERNING A FILING UNDER 35 USC 371**

International Application No.:

PCT/JP00/06129

International Filing Date:

September 8, 2000

Priority Date Claimed:

September 9, 1999

Title of Invention:

MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND

METHOD FOR REGENERATING CARDIOVASCULAR TISSUE

Applicant(s) for DO/EO/US:

Shinichiro Morita, Toshiharu Shin'oka and Yasuharu Imai

- Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: This is a FIRST submission of items concerning a filing under 35 USC 371. 1. (X) 2. This express request to begin national examination procedures (35 USC 371(f)) at any time rather (X) than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1). 3. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 4. (X) A copy of the International Application as filed (35 USC 371(c)(2)) a) is transmitted herewith (required only if not transmitted by the International Bureau). b) (X) has been transmitted by the International Bureau. c) (X) a copy of Form PCT/1B/308 is enclosed. d) is not required, as the application was filed in the United States Receiving Office () (RO/US). 5. (X) A translation of the International Application into English (35 USC 371(c)(2)). 6. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3)a) 0 are transmitted herewith (required only if not transmitted by the International Bureau). b) 0 have been transmitted by the International Bureau. have not been made; however, the time limit for making such amendments has NOT c) 0 expired. d) (X) have not been made and will not be made. 7. (X) International Application as published - face sheet only. 8. (X) International Search Report.
- 9.

(X)

- 10. (X) Verification of a translation.
- 11. (X) International Application as published - face sheet only.
- 12. (X) Two (2) sheets of photo drawings.

PCT Request Form.

U.S. Application No.

International Application No. PCT/JP00/06129

JC13 Rec'd PCT/PTO 0.7 MAR 2002

Attorney Docket No. SAEG108.001APC

Page 2

Date: March 7, 2002

13. (X) A return prepaid postcard.

13.	(X)	A return prepaid postcard.				
14.	(X)	The following fees are submitted:				
						FEES
			BASIC FEE			\$890
CLAIMS			NUMBER FILED	NUMBER EXTRA	RATE	
Total Claims			11 - 20 =	0 ×	\$18	\$0
Indep	endent C	aims	1 - 3 =	0 ×	\$84	\$0
			TOTAL OF AB	OVE CALCULATION	NS \$890	
			TOTAL FEES	ENCLOSED		\$890
15.	(X)	The fee for later submission of the signed oath or declaration set forth in 37 CFR 1.492(e) will be paid upon submission of the declaration.				
16.	(X)	A check in the amount of \$890.00 to cover the above fees is enclosed.				
17.	(X)	The Commissioner is hereby authorized to charge only those additional fees which may be required, now or in the future, to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410.				

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Katsuhiro Arai Reg. No. 43,315

Customer No. 20,995

H:\DOCS\KOA\KOA-1885 DOC 030602

SAEG108.001APC

15

20

2 prt

)

PATENT

-1-

DESCRIPTION

MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND METHOD FOR REGENERATING CARDIOVASCULAR TISSUE

TECHNICAL FIELD

The present invention relates to a matrix for culturing cardiovascular cells to regenerate cardiovascular tissue and a method for regenerating cardiovascular tissue such as an artificial blood vessel, cardiac valve, pericardium, etc.

10 BACKGROUND ART

In the field of artificial vessels, for instance, those made of non-bioabsorbable polymers are widely used. An artificial vessel (GORE-TEX), for example, is used most frequently in a clinical field. Such non-bioabsorbable artificial vessel is excellent in physical properties; however, because of the non-bioabsorbability, it remains in vivo as a foreign body for a long period of time after implantation. Further, when the non-bioabsorbable artificial vessel is implanted into the body of a child, another surgery for replacement is necessary since the non-bioabsorbable artificial vessel does not expand with the growth of the autogeneous blood vessel.

A tissue regeneration method employing tissue engineering techniques has recently been developed, wherein cells of autogeneous tissue are seeded and

cultured on a scaffold made of a bioabsorbable polymer so as to regenerate the autogeneous tissue. There have been published quite a few research reports of the tissue regeneration method applied to skin regeneration (M. L. Cooper, L. F. Hansbrough, R. L. Spielvogel, et al.: In 5 vivo optimization of a living dermal substitute employing cultured human fibroblasts on a biodegradable polyglycolic acid or polyglactin mesh. Biomaterials, 12:243-248, 1991) and cartilage regeneration (C.A. Vacanti, R. Langer, et 10 al.: Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. Plast. Reconstr. Surg., 88:753-759, 1991).

If a blood vessel can be regenerated in the same manner as described above, growth of the regenerated blood vessel is expected since it is regenerated by using autogeneous tissue and no longer necessitates the use of anti-coagulants.

15

20

25

An object of the present invention is to provide a matrix which allows cells to sufficiently adhere thereto, provides an optimum scaffold for cell proliferation, maintains satisfactory blood flow resistance in vivo till autogeneous tissue is regenerated, and is ultimately decomposed and absorbed in vivo.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a photograph showing a cross-sectional

view of a vascular regeneration matrix according to the present invention.

Fig. 2 is a photograph showing a plan view of a vascular regeneration matrix according to the present invention.

5

20

25

Fig. 3 is a photograph of the angiogram recorded at the $3^{\rm rd}$ postoperative month.

DISCLOSURE OF INVENTION

Basic requirements for the matrix for culturing cardiovascular cells to regenerate cardiovascular tissue are an ability to allow cells seeded thereon to adhere firmly thereon and a bioabsorbability which enables the matrix to be absorbed in vivo when a blood vessel is regenerated. A sponge is considered to be the optimum material to fulfill the above requirements.

In the case of using the matrix for regenerating a blood vessel, the matrix is required to maintain an enough strength to endure a blood flow for a certain period of time after implantation till the blood vessel is regenerated in vivo.

The inventors found that the above object is achieved by strengthening, with a reinforcement made of a bioabsorbable material, a sponge made of a bioabsorbable material which is an optimum scaffold for cell proliferation and excellent in cell adhesiveness.

The present invention provides a matrix for culturing cardiovascular tissue and a method for regenerating cardiovascular tissue of the following items.

Item 1. A matrix for culturing cardiovascular cells to regenerate cardiovascular tissue comprising a sponge made of a bioabsorbable material and a reinforcement made of a bioabsorbable material.

Item 2. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1, wherein the bioabsorbable material is at least one member selected from the group consisting of polyglycolic acid, polylactic acid (D form, L form, DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form) copolymer, glycolic acid-caprolactone form, DL copolymer, lactic acid (D form, L form, DL form) caprolactone copolymer, poly(p-dioxanone) and the like.

10

15

20

25

Item 3. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1 for use in regenerating an artery, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

Item 4. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1 for use in regenerating a vein, wherein the sponge comprises a lactic acid-caprolactone copolymer and the

reinforcement comprises polyglycolic acid.

5

10

20

Item 5. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1 for use in regenerating a cardiac valve or a pericardium, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

Item 6. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1, wherein the sponge has a pore diameter of about 5 µm to about 100 µm.

Item 7. A method for regenerating cardiovascular tissue comprising seeding cells on the matrix of item 1 and culturing the cells.

Item 8. The method for regenerating cardiovascular tissue according to item 7, wherein the cardiovascular tissue to be regenerated is a blood vessel.

Item 9. The method for regenerating cardiovascular tissue according to item 7, wherein the cardiovascular tissue to be regenerated is a cardiac valve.

Item 10. The method for regenerating cardiovascular tissue according to item 7, wherein the cardiovascular tissue to be regenerated is a pericardium.

Item 11. The method for regenerating 25 cardiovascular tissue according to item 7, wherein the

cells to be seeded are a mixed cell culture of two or three different kinds selected from the group consisting of endothelial cells, smooth muscle cells and fibroblasts.

According to the invention, it is preferable that regeneration of cardiovascular tissue be conducted by seeding cells to a matrix for culturing cardiovascular cells and embedding the matrix in vivo to regenerate cardiovascular tissues in vivo.

5

15

20

25

Examples of bioabsorbable material include 10 polyglycolic acid, polylactic acid (D form, L form, DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form) copolymer, glycolic acid-caprolactone copolymer, lactic acid (D form, ${
m L}$ form, DLcaprolactone copolymer, poly(p-dioxanone) and the like.

Examples of cardiovascular tissue include blood vessels, cardiac valves, the pericardium and the like.

The matrix of the invention is obtained by strengthening a sponge made of a bioabsorbable material with a reinforcement (in the form of a fiber, nonwoven fabric or film) made of a bioabsorbable material. There is no limitation on the bio-absorbable materials to be used for the sponge and the reinforcement. In the case of preparing the matrix for regenerating a blood vessel, a sponge made of a lactic acid-caprolactone copolymer may be combined with a reinforcement made of polylactic acid when

the blood vessel is an artery, and the same sponge may be combined with a reinforcement made of polyglycolic acid when the blood vessel is a vein. Further, in the case of regenerating a cardiac valve or the pericardium, a sponge made of a lactic acid-caprolactone copolymer may be combined with a reinforcement made of polylactic acid.

The sponge has pores each having such a pore size that cells can suitably be adhered thereto to proliferate and that no blood leakage is caused when the matrix comprising the sponge is implanted cardiovascular tissue. The pore size may typically be about 1 mm or less, preferably about 5-100 μm . The shape of the matrix may be cylindrical when the cardiovascular tissue to be regenerated is a blood vessel or may be plane when the cardiovascular tissue to be regenerated is a cardiac valve or the pericardium. In the case regenerating a blood vessel, the length and inside diameter of the matrix may be adjusted depending on the target blood vessel. The thickness of the matrix is chosen depending on the desired period for bio-absorption or ease of suturing. The thickness may typically be about 5 mm or less, preferably from about 500 μm to about 2 mm.

For preparation of the sponge, the following alternative processes, among others, are available.

25 (1) Lyophilization process

5

10

15

A substrate polymer solution is poured in a mold, frozen, and, then lyophilized. According to the freezing temperature and polymer concentration, sponges having various pore diameters are obtained (described in Examples).

(2) Elution process

5

10

15

20

25

A water-soluble material is mixed with the substrate polymer solution and, after drying, the water-soluble material is washed out with rinse water. The resultant sponge has a pore diameter corresponding to the particle size of the water-soluble material used. In the present case, sucrose can be used with advantage.

The reinforcement must have a greater strength than the sponge. The reinforcement can be selected from among a fiber, nonwoven cloth, film and so on.

The reinforcement is preferably integrated with the sponge and can be located either on the interior surface, inside, or exterior surface of the sponge. However, since the interior surface of the sponge is involved in the adhesion of vascular endothelial cells, it is preferably situated inside or on the exterior surface, although the interior surface may be optionally used.

As to the cells to be seeded, substantially the same kinds of cells are used for various cardiovascular tissues in common. Thus, they are endothelial cells,

smooth muscle cells and fibroblasts, and a mixed cell culture of two or three different kinds of cells can be mentioned by way of example. Tissue construction is carried out using such mixed culture cells.

5 The cultural conditions for the cells to be used and the seeding method are described below.

A. Cell isolation, culture, and propagation

15

20

25

The vascular tissue isolated in а sterile environment is immersed in a cell culture medium and washed with phosphate-buffered saline in a clean bench. 10 Then, on a Petri dish, the tissue is cut into pieces using a surgical knife according to the simple explant technique. $1-2 \text{ mm}^2 \text{ are}$ Tissue pieces sized about distributed uniformly on the dish and after about 20 minutes, when the tissue pieces have intimately adhered to the bottom of the dish, a culture medium is added. As the culture medium, Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10 % fetal calf serum and 1 % antibiotics solution (Lglutamine 29.2 mg/ml, penicillin G 1000 U/ml, streptomycin sulfate $10,000 \mu g/ml$) is used. The mixed cells of endothelial cells and fibroblasts begin to migrate from the tissue pieces on the dish after 5-7 days, forming mixed-cell colonies around the explants in a further one After another 2-3 weeks, the mixed cells become confluent on the dish. Immediately, a passage is made

using 0.25 % trypsin and the culture in a 75 cm² culture flask is started. Generally when the growth in this flask has become confluent, about 2x10⁶ cells are available. Cell culture is performed under an atmosphere comprising 5 % CO₂ and 21 % O₂ and continued until 10x10⁶ cells have been obtained. While the culture medium is renewed every 4-5 days, the resultant of a preliminary experiment has shown that the doubling time of cells is about 48 hours. Incidentally, the counting of cell population during the course is carried out by the classical exclusion method using Trypan Blue.

B. Cell sorting and endothelial cell purification

At the stage when the mixed cells have become confluent and a reasonable number of cells is obtained, endothelial cells are sorted out from among the mixed 15 cells using FACS according to the following protocol. Thus, Dil-acetylated LDL (fluorescent dye marker; product of Biomedical Technologies) (briefly, Dil-Ac-LDL) is added to the mixed cell culture at a concentration of 1 $\mu g/ml$, 20 followed by 24-hour incubation. This marker is taken up intracellularly through a scavenger pathway specific to endothelial cells and macrophages. After 24 hours, the cells are trypsinized to prepare a mixed cell suspension and sorting is performed using a cell sorter (FACS machine; product of Bectin Dickenson). According to the 25

size and emission of fluorescence, the cells are sorted into Dil-Ac-LDL-positive cells and Dil-Ac-LDL-negative cells. After the sorting, these types of cells are independently cultured and the culture is continued until 2×10^6 endothelial cells are obtained.

C. Tissue construction

5

10

15

The first step of tissue construction comprises seeding cells in vitro. Specifically, a biodegradable culture matrix is seeded with about 1×10^6 cells/cm² of Dil-Ac-LDL-negative fibroblasts.

Immediately following the seeding of a concentrated cell suspension on the matrix, the system is allowed to stand on the culture dish in a clean bench for 30-60 minutes, and thereafter about 50 ml of a culture medium is added. The culture medium is renewed every day as a rule and after 7 days, that is, one day before surgical transplantation, a further seeding is performed with a suspension of endothelial cells (about 2×10^6 cells), whereby a monolayer of endothelial cells is obtained.

The above steps A-C show the cell isolation, culture and seeding procedures for the construction of a heart valve, a pericardium, or a blood vessel.

BEST MODE FOR CARRYING OUT THE INVENTION

The following examples are further illustrative of the present invention.

Example 1

· Construction of a vascular regeneration matrix

A glass test tube (10 mm in outside diameter) was wrapped around with a plain-weave cloth of poly-L-lactide fiber (photograph) in a double-cylindrical form. This assembly was set in a mold (12 mm in inside diameter) and a solution of L-lactide-caprolactone copolymer (50:50) in dioxane (5%) was poured into the clearance, frozen and then lyophilized.

The cylindrical vascular prosthesis thus obtained was a cellular substrate reinforced with a fibrous material (Figs. 1 and 2).

· Cell culture

Through a small skin incision, a peripheral vein segment, about 5 mm long, was excised in a sterile environment and immediately immersed in the tissue culture medium. Cell isolation was carried out by the simple explant technique. As the cell culture medium, the standard cell culture medium DMEM mentioned above was used, and the medium was renewed every 2-3 days.

· Seeding of cells

25

The matrix prepared above was seeded with about 1×10^6 cells/cm² of a mixed culture of endothelial cells and fibroblasts and the culture was continued for about 1 week until the matrix surface had been completely covered

-13-

with the cells.

· Animal experiment

The vascular prosthesis constructed as above was transplanted in the inferior vena cava of a young dog. As a result, no obliteration by rupture was found and a good patency could be verified angiographically at the 3rd postoperative month (the angiograph in Fig. 3). Thoracotomy at 6 months revealed regeneration of autogenous blood vessel in agreement with the transplantation site.

In contrast, the matrix not reinforced with poly-L-lactide fiber ruptured in one week after substitution and the experimental animal succumbed to sudden death.

10

Claims

1. A matrix for culturing cardiovascular cells to regenerate cardiovascular tissue comprising a sponge made of a bioabsorbable material and a reinforcement made of a bioabsorbable material.

- 2. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1, wherein the bioabsorbable material is at least one member selected from the group consisting of polyglycolic acid, polylactic acid (D form, L form, DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form, DL form, DL form) copolymer, glycolic acid-caprolactone copolymer, lactic acid (D form, L form, DL form)-caprolactone copolymer and poly(p-dioxanone).
- 3. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating an artery, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.
- 4. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating a vein, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polyglycolic acid.
- 5. The matrix for culturing cardiovascular

cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating a cardiac valve or a pericardium, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

5

15

- 6. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1, wherein the sponge has a pore diameter of about 5 μm to about 100 μm .
- 7. A method for regenerating cardiovascular tissue comprising seeding cells on the matrix of Claim 1 and culturing the cells.
 - 8. The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a blood vessel.
 - 9. The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a cardiac valve.
- 10. The method for regenerating cardiovascular 20 tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a pericardium.
 - 11. The method for regenerating cardiovascular tissue according to Claim 7, wherein the cells to be seeded are a mixed cell culture of two or three different kinds selected from the group consisting of endothelial

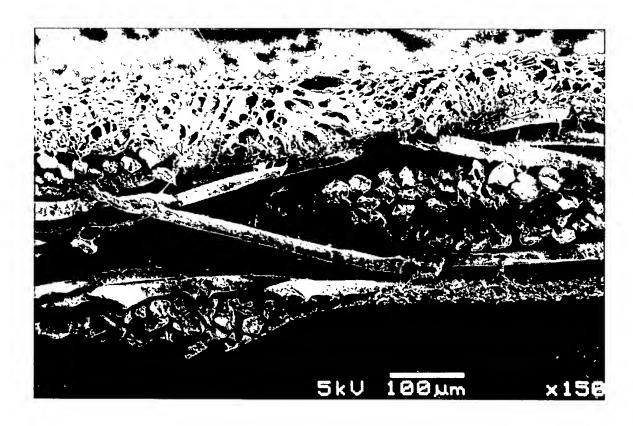
cells, smooth muscle cells and fibroblasts.

Abstract

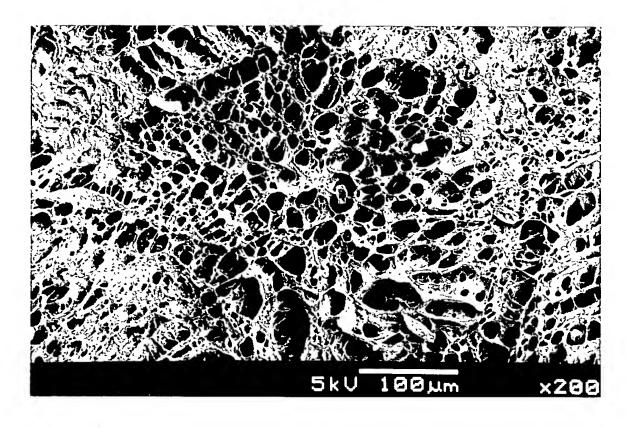
Materials for culturing cardiovascular tissues wherein a sponge made of a bioabsorbable material is reinforced with a reinforcement made of a bioabsorbable 5 material.

1 / 2

F | G . 1



F 1 G . 2



2 / 2

rykani s

F 1 G . 3



Attorney's Docket No.	SAEG108.	001APC
-----------------------	----------	--------

DECLARATION AND POWER OF ATTORNEY - USA PATENT APPLICATION

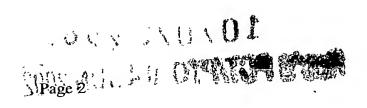
As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND METHOD FOR REGENERATING CARDIOVASCULAR TISSUE the specification of which: (a) is attached hereto; or (b) ₩. was filed on __ March 7, 2002 _ as Application No. __10/070938 _____ or Express Mail No., as Application No. not yet known ___ _and was amended _(if applicable); or on_ X(c) was described and claimed in PCT International Application No. PCT/JP00/06129 __filed on <u>September 8, 2000</u> and as amended under PCT Article 19 on___ (if any) and/or under PCT Article 34 on ___ (if any). I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above; I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56; I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent, design or inventor's certificate or any PCT international application(s) listed below and have also identified below any foreign application(s) for patent, design or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed for the same subject matter having a filing date before that of the application(s) of which priority is claimed: PRIOR FOREIGN APPLICATION(S)

COUNTRY (OR INDICATE IF PCT)				CLAIMED U.S.C. § 119
Japan	1999-255803	09/09/1999	₩ YES	№□
			☐ YES	NO □
			☐ YES	NO 🗆
			□ YES	NO 🗆

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below, and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application:



Attorney's Docket No. SAEG108.001 APC

	Prior U.S.A. Application(s)		••			
	Application No.:	Filing Date:	Status:			
	POWER OF ATTORNEY: I hereby appoint the registrants of Knobbe, Martens, Olson & Bear, LLP, 620 Newport Center Drive, Sixteenth Floor, Newport Beach, California 92660, Telephone (949) 760-0404, Customer No. 20,995.					
	on information and belief are that willful false statements a 1001 of Title 18 of the United application or any patent issue	believed to be true; and further and the like so made are punished d States Code and that such will ded thereon.	ny own knowledge are true and that all statements made or that these statements were made with the knowledge hable by fine or imprisonment, or both, under Section Iful, false statements may jeopardize the validity of the			
50	Full name of sole or first inve	ntor: <u>MORITA</u> Shinichirc				
	Inventor's signature	Day 21st	Month May Year 2002			
	Residence (city and country):	Ayabe-shi, Kyoto 623	-0051, Japan 9 X			
	Citizenship: Japan	Poscarah C Dava Lormant	Department of GUNZE LIMITED, nachi, Ayabe-shi, Kyoto 623-0051, Japan			
	Post Office Address:	illouto, illoutu oliila	delle, in dee energy and the energy and			
00	Full name of second inventor:	SHIN'OKA Toshiharu				
	Inventor's signature	hinoka Day 21st	Month May Year 2002			
	Residence (city and country):	Shinjuku-ku, Tokyo 1	62-0054, Japan			
	Citizenship: Japan	TOKYO MENTENTE MENTENT.				
	Post Office Address:	10.000				
W.	Full name of third inventor:	IMAI Yasuharu	Month May Year 2002			
	Inventor's signature Jasuh	Day 21st	Month May Year 2002			
	Residence (city and country):	Shinjuku-ku, <u>Tokyo</u> 1	.62-0054, Japan			
	Citizenship: Japan	TOKYO WOMEN'S MEDICAL	TINTVERSTTY			
•	Post Office Address: 8-1,	Kawada-cho, Shinjuku-	ku, Tokyo 162-0054, Japan			
•	Send Correspondence To: KNOBBE, MARTENS, OLS Customer No. 20,995 PF-33	ON & BEAR, LLP				